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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/026,586	12/18/2001	Manoj Kumar	GC558D1	9370

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GENENCOR INTERNATIONAL, INC.
ATTENTION: LEGAL DEPARTMENT
925 PAGE MILL ROAD
PALO ALTO, CA 94304

EXAMINER	
RAO, MANJUNATH N	

ART UNIT	PAPER NUMBER
1652	

DATE MAILED: 03/30/2004

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/026,586	KUMAR, MANOJ	
	Examiner	Art Unit	
	Manjunath N. Rao, Ph.D.	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *10 November 2003*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *20-29 and 41-46* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *20-29 and 41-46* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Claims 20-29, 41-46 are currently pending and are present for examination.

Applicants' amendments and arguments filed on 11-10-03, have been fully considered and are deemed to be persuasive to overcome the rejections previously applied. Rejections and/or objections not reiterated from previous office actions are hereby withdrawn. Specifically, Examiner has withdrawn the rejections under 35 U.S.C. 112, 2nd paragraphs in view of claim amendments.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 20-25 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a recombinant yeast wherein said yeast is a member of Cryptococcaceae such as *Candida blankii* or *Cryptococcus dimennae* which are capable of utilizing KLG as its sole source of carbon to produce ascorbic acid or ascorbic acid stereoisomer, wherein said yeasts comprise either one or both of a heterologous nucleic acid encoding an oxidative enzyme and a reducing enzyme associated with production of ascorbic acid, does not reasonably provide enablement for claiming any or all yeasts (including variants and mutants) with the characteristic property of utilizing KLG as sole carbon source to produce ascorbic acid. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

Factors to be considered in determining whether undue experimentation is required, are summarized in *In re Wands* (858 F.2d 731, 8 USPQ 2nd 1400 (Fed. Cir. 1988)) as follows: (1) the breadth of the claim(s), (2) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, and (7) the predictability or unpredictability of the art.

Claims 20-25 are so broad as to encompass any yeast(s) capable of utilizing KLG as its sole source of carbon. The scope of the claims is not commensurate with the enablement provided by the disclosure with regard to the extremely large number of yeasts that are broadly encompassed by the claims.

First of all applicants are claiming all or any yeast that is capable of utilizing KLG to produce ascorbic acid. The term yeasts includes an enormously large group of organisms many of which may or may not have the above capability. Thus by claiming "yeasts", applicants are claiming an enormously large group of microorganisms for which a representative number itself would run into several hundreds . Next, applicants have shown that the above characteristic feature is existent only in the two yeast species which produce a specific enzyme that imparts the capability of utilizing KLG as sole carbon source, and since applicants have not shown that this capability is exhibited by all species and strains yeasts, the claims encompass yeasts that are capable of utilizing KLG as sole source of carbon by other mechanisms existing in nature. Furthermore, it is also well known in the art that the different strains within a single species or different species within a single genus can vary in their physical and biochemical characteristics. For example, not all strains of *E.coli* are known to cause food poisoning due to production of a

heat stable toxin. As the above characteristic (i.e., use of KLG as sole carbon source) of the yeast is dependent on the enzymes produced by the microorganisms, predictability of existence of pathways for use of KLG as sole carbon source requires a knowledge of and guidance of the ways to identify and characterize specific microorganism in which the enzyme proteins' structure relates to its function. However, in this case the disclosure is limited to the few microorganisms which have been deposited in a culture collection.

While microbial isolation and identification techniques are known, it is not routine in the art to screen for multiple strains, as encompassed by the instant claims, and a reasonable expectation of success in obtaining the desired activity/utility are limited and the results of such identifications are unpredictable.

Examiner has concluded that the specification does not support the broad scope of the claims which encompass all yeasts with the above characteristics based on the following analysis: (A, Breadth of the claim) Applicants have not shown that the assay they provide to identify the yeast is suitable to test any yeast. The assay they have provided is mainly for the two specific species. While applicants may argue that the assay they have provided is enough to identify any yeast, Examiner disagrees with such an argument. This is because, among yeasts there are different sub groups which are highly diverse and no single method or assay would apply to all of them. B. (Nature of the invention) Applicants have not shown that the mechanism involved (enzymatic pathway) in the use of KLG as sole source of carbon is universal in all yeasts. Applicants may argue that the assay they have provided is quite enough to identify any yeast. Examiner respectfully disagrees. This is because of the nature of invention. It is well known in the art of microbiology that microbes invariably have alternate

pathways for making or breaking a compound. While applicants may claim that they have provided an assay it is highly likely that alternate pathway may be present in nature, and because of this the assay provided by the applicants may not work on all yeasts. C. (State of the prior art) The prior art is not rich in the above type of inventions i.e., the subject matter of the above invention is practiced by a small group of inventors and there is no information regarding the capability of all the different types of yeasts etc. in utilizing KLG as sole carbon source. Applicants have also not shown the above ability in any other yeast. Therefore, there are no examples of the above invention in the prior art. D. (level of ordinary skill) The specification does not provide a universal method that can be used by any one skilled in the art. This is because, while the specification simply provides the assay method that can be performed by one skilled in the art familiar with *Candida* and *Cryptpococcus*, applicants have not provided enough guidance as to how one interested in isolating other members of yeast group would use the assay method for isolating the claimed yeasts. The above group of yeast have special culture requirements and applicants have not provided enough guidance such that their method can be used for testing any member of the yeast. E (level of predictability in the art/ amount of direction provided by the inventor/existence of working examples) While predictability is quite straight forward in mechanical and electrical arts, it is highly unpredictable in bioscience arts. This aspect has been well understood in all court decisions. Therefore, while applicants may argue claim that the assay provided would be able to identify any yeast, because of the complexity of the microbial cells, an assay that is applicable for one type of microorganism may be completely useless for another type. For example it has been acknowledged in *In re Fisher*, 427 F.2d 833, 839,166 USPQ 18, that in cases involving unpredictable factors, such as most chemical reactions

and physiological activity, more may be required and in applications directed to inventions in art where the results are unpredictable, the disclosure of a single species usually does not provide an adequate basis to support generic claims, *In re Soll*, 97 F.2d 623, 624, 38 USPQ 189, 191 (CCPA 1938). F.(quantity of experimentation needed to make the invention) The specification provides insufficient guidance as to which of the essentially infinite possible choices is likely to be successful. This is because applicants have not provided a method or methods that can be used to identify any microorganism.

Thus, applicants have not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims broadly including any yeast with the characteristic of utilizing KLG as sole source of carbon. The scope of the claims must bear a reasonable correlation with the scope of enablement (*In re Fisher*, 166 USPQ 19 24 (CCPA 1970)). Without sufficient guidance, identification of a microorganism having the desired biological characteristics is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. See *In re Wands* 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988).

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 20-29, 41-43 are rejected under 35 U.S.C. 103(a) as being unpatentable over Murakawa et al. (Agric. Biol. Chem., Vol 41(9):1799-1800), Hardy et al. (US 4,945, 052 issued Jul 31, 1990) and Anderson et al. (US 5,032,514, 7-16-1991). Claims 20-29, 41-43 in this instant application are drawn to a recombinant yeast capable of utilizing keto-L-gulonic acid to produce ascorbic acid or an ascorbic acid stereoisomer comprising or expressing a heterologous gene for an oxidative and reductive enzyme such as 2-keto-D-gluconic acid dehydrogenase and 2,5 DGK reductase respectively, rendering the yeast capable of utilizing 2-keto-L-gulonic acid (2-KLG), an intermediate in the biosynthesis of ascorbic acid and finally bioconverting KLG to ascorbic acid, wherein said yeast is a member of Cryptococcaceae, belonging to genus *Candida* or *Cryptococcus* specifically *C.blankii* or *C.dimennae* .

Murakawa et al. teach the production of ascorbic acid from non-recombinant yeasts using wide variety of sugars including glucose. However, the yields appear to be low. Thus, it appears that it was well known in the art that 2,3-DKG occurs among yeasts and that they are capable of producing ascorbic acid. Andersen et al. teach a metabolic pathway for engineering an increased production of ascorbic acid intermediates by using recombinant technology by transfer of genes responsible for the bioconversion of a six carbon sugar such as glucose to 2-KLG which is next oxidized to ascorbic acid using the very same enzymes taught in this instant application. (See entire document, specifically, column 1, lines 55-69 and column 2, lines 61-66; column 3 lines 33-36, 43-48, 63-69; column 4, lines 26-32, 43-60; column 5 line 43; column 11, lines 15-43, column 13, lines 26-3637-65, column 18-19, example 4). However, the reference does not teach the utilization of yeasts for the fermentative method or the bioconversion method. The reference does teach that the recombinant technique can be used using any appropriate host

cells (see column 7, lines 63-68 and column 8, lines 1-8). Hardy et al. teach the production of vitamin C precursor, 2, 5-DKG, in genetically modified microorganisms including several bacteria, fungi and yeasts (see column 5, last para) by transforming yeast host cells using a vector expressing the enzyme required for converting 2,5DKG to 2-KLG. The reference teaches recombinant methods and suggests the use of a list of microorganisms and mammalian cells as host cells.

With the above references in hand, it would have been obvious to one skilled in the art at the time the invention was made to combine the teachings of Murakawa et al. with that of Andersen et al. or Hardy et al. to engineer a yeast cell such as a *Candida*, capable of utilizing 2KLG and convert it into ascorbic acid. Murakawa et al. teach that yeasts are capable of producing ascorbic acid. Hardy et al. teach the use of yeasts as host organisms. Because the production of ascorbic acid is low among yeasts, one skilled in the art would be motivated to combine the teachings of Murakawa et al. with that of the molecular biological techniques of Andersen et al. to develop yeasts capable of utilizing glucose more efficiently and produce ascorbic acid in large amounts such that ascorbic acid can be produced on a commercial scale in a one pot synthesis. Furthermore, Andersen et al. reference also teach that one would be motivated to do this as they are several advantages of having a yeast, a well known industrial microorganism, capable of producing vitamin C, to produce large amounts of ascorbic acid which has a huge demand in food and pharmaceutical industry. One would have a reasonable expectation of success since Murakawa et al. demonstrate the production of vitamin C from yeasts which are well known as fermentation work horse and Andersen et al. provide the entire metabolic machinery and the genes and enzymes necessary for doing the same and Hardy et al.

demonstrate that yeasts can be used as host cells to introduce vectors for vitamin C precursor enzymes.

Therefore it would have been *prima facie* obvious to one of ordinary skill in the art to have performed the claimed invention.

In response to the previous Office action, applicants have traversed the above rejection arguing that Examiner has not shown a prima facie obviousness and that there is no disclosure or suggestion found in the cited references that either alone or in combination for a recombinant yeast with the claimed characteristics. While applicants do agree that parts of the invention can be found the invention as a whole is not made obvious by the combination of the references. Examiner respectfully disagrees with such an argument by the applicants to be persuasive to overcome the above rejection. This is because, applicants appear to ignore the specific suggestion and teaching by Hardy et al. that yeasts can be used in the conversion of a carbon source such as glucose to 2-KLG in a single fermentation step. Examiner takes the position that this reference is the critical reference which not only suggests but also provides the motivation to combine the references cited by one skilled in the art to arrive at the above invention. Applicants appear to ignore that fact that hardy et al. suggests yeasts as one of the host cell that can be used and focus their argument that the reference of hardy is all about the use of Erwinia, a fungus. As applicants have admitted the parts of the above invention is indeed taught by all the recited references and the single reference of Hardy et al. provides the suggestion and the motivation to combine the teachings to arrive at the above invention. Therefore contrary to applicant's argument examiner maintains that the above references render claims 20-29, 41-43 *prima facie* obvious to those skilled in the art.

Claims 44-46 are rejected under 35 U.S.C. 103(a) as being unpatentable over Murakawa et al. (Agric. Biol. Chem., Vol 41(9):1799-1800), Hardy et al. (US 4,945, 052 issued Jul 31, 1990) and Anderson et al. (US 5,032,514, 7-16-1991) as applied to claims 20-29 above, and further in view of Saito et al. (Appl. Environ. Microbiol., 1997, Vol. 63(2):454-460). Claims 44-46 are drawn to a recombinant yeast capable of utilizing keto-L-gulonic acid to produce ascorbic acid or an ascorbic acid stereoisomer comprising using L-sorbitol by expressing a heterologous gene for an oxidative and reductive enzyme such as L-sorbose dehydrogenase, to produce KLG from sorbitol.

The references of Murakawa et al., Hardy et al. and Anderson et al. have all been discussed above. Saito et al. disclose the cloning of the genes coding for L-sorbose dehydrogenase and also teach the use of a recombinant bacteria transformed with said gene capable of using sorbitol for production of KLG. However, the reference does not teach a recombinant yeast strain comprising the said genes.

Combining the above references it would have been obvious to one of ordinary skill in the art to transform a yeast cell with the sorbitol dehydrogenase activity taught by Saito et al. in order to obtain a recombinant yeast capable of using sorbitol to produce KLG and further convert the same to ascorbic acid. One of ordinary skill in the art would have bee motivated to do so in order to use a cheap carbon source, sorbitol, for production of ascorbic acid. One of ordinary skill in the art would have a reasonable expectation of success since all the references except for Saito et al. teach the making of a yeast or a recombinant yeast for ascorbic acid production and Saito et al. specifically teach the use of sorbitol dehydrogenase genes for achieving the same goal using a cheap carbon source.

Therefore it would have been *prima facie* obvious to one of ordinary skill in the art to have performed the claimed invention.

Here again, applicants have argued that as the references used for rejection of claims 20-29 and 41-43 have been argued not to render the claims as obvious, the combination of the same references with Saito et al. also does not render claims 44-46 as obvious. However, Examiner respectfully disagrees with such an argument and continues to maintain the above rejection based on the above rejection of claims 20-29 and 41-43.

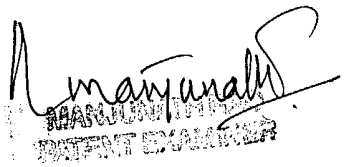
This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(f) or (g) prior art under 35 U.S.C. 103(a).

Conclusion

None of the claims are allowable.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Manjunath N. Rao, Ph.D. whose telephone number is 571-272-0939. The examiner can normally be reached on 6.30 a.m. to 3.00 p.m. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathapura

Achutamurthy can be reached on 571-272-0928. The fax phone numbers for the organization where this application or proceeding is assigned are 703-308-4242 for regular communications and 703-308-4242 for After Final communications. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is 571-272-1600.



Manjunath N. Rao
March 29, 2004